REMARKS

Claims 24-27, 29 and 31 have been canceled, without prejudice. The cancellation of these claims obviates the rejection of these claims.

New Claims 32-34 read on the elected invention and have been added to further claim the invention. New Claim 32 requires that the claimed antibody specifically binds both the tetrapeptide (SEQ ID NO:2) and one of the pentapeptides (SEQ ID NO:1 or SEQ ID NO:4). Support for Claims 32-34 can be found in the specification, for example, it is reported that a preferred embodiment of the invention employs a binding pair member having affinity to one or more of the polypeptides of SEQ ID NO:1, 2 and 4 in immunochemical procedures to detect the occurrence of the magnesium binding defect. [0024] Extensive detail of how to generate polyclonal and monoclonal antibodies is provided in the specification, for example at paragraphs [0024]-[0036] and for generating and selecting hybridomas for monoclonal antibody production [0037]-[0044]. Thus, it is implicit in this embodiment that, the antibody may cross-react with the tetrapeptide and pentapeptides. It is believed that none of these amendments constitute new matter and their entry is requested.

Rejection Under 35 U.S.C. § 112

The Examiner has rejected Claims 1, 2, 7-10, 28 and 20-31 under 35 U.S.C. 112, first paragraph, on the basis that the specification is not fully enabling, commensurate with the scope of the claims. The cancellation of Claims 24-27, 29 and 31 obviate the rejection of these claims.

The Examiner has acknowledged that the instant specification is enabling for an antibody that specifically binds the sequence of SEQ ID NO:1 and SEQ ID NO:4. However, Examiner asserts that based on the teachings of Couraud et al., the specification is not enabling for an antibody that specifically binds SEQ ID NO:2. Applicant submits that the specification would

enable one of ordinary skill in the art to practice the full breadth of the claimed invention and respectfully traverses the Examiner's rejection.

Only routine experimentation is required to generate an antibody that binds to the peptide of SEQ ID NO:2.

The teachings of the Harlow et al. publication (1988) referred to in the Final Office Action (page 5) have been revised and replaced by Harlow et al. (1999) (Using Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory Press). Harlow et al. (1999), therefore describes at least minimum knowledge in the art as of the filing date of the instant application. Applicant provides certain relevant sections of this publication.

> The region of an antigen that interacts with an antibody is defined as an epitope. An epitope is not an intrinsic property of any particular structure, as it is defined only by reference to the binding site of an antibody. The size of an epitope is governed by the size of the combining site. From X-ray studies of the structures of cocrystals between small antigens bound to antibodies, the size of the combining site was though to be relatively small. . . .Later work using larger antigens shows that the area of these antigens in close apposition to the antibody can be quite large, occupying as much as 500-700 A^2 and often involving contacts with multiple CDRs, and many times establishing contact with all six. Although these studies have shown that epitopes can be much large than originally thought, it is still clear that high-affinity antibodies can be raised to small epitopes. (page 25)

> There are many potential methods for mapping and characterizing the location of epitopes on proteins, ranging from solving the crystal structure of the antibody-antigen complex to analysis of vast libraries of random peptide sequences. . . Three of the simplest, most widely applicable, and most robust assays are competition assays, gene expression assays, and synthetic peptide-based assays. (page 384)

Table 11.2 (page 384) of Harlow et al. teaches that synthetic peptide based assays can be used to map linear epitopes of 3-15 amino acids.

Harlow et al. (1999) teaches use of monoclonal antibodies in immunoprecipitation technique (page 226-227) noting that an epitopes are "often comprised of only 4 or 5 amino acids" (second paragraph, pg. 227).

Applicant thus urges that only routine experimentation using art recognized methods would be required to generate the claimed antibodies. The fact that some amount of work must be performed to reach a successful end does not mean that a claimed composition is not enabled.

> "Enablement is not precluded by the necessity for some experimentation such as routine screening. However, experimentation needed to practice the invention must not be undue experimentation. 'The key word is 'undue,' not 'experimentation.'
>
> The determination of what constitutes undue experimentation in a given case requires the application of a standard of reasonableness, having due regard for the nature of the invention and the state of the art. ... The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." *In re Wands*, 8 U.S.P.Q.2d 1400, 1404.

As noted in Wands, the need for routine screening is allowable and does not mean that an invention is not enabled. In the instant case, the fact that antibody positive hybridomas need to be screened for antibodies for the desired reactivity does not mean that the claimed antibodies are not enabled.

Furthermore, objective enablement, not actual reduction to practice, is all that is required, as stated by the court in *Fiers v. Revel*, 984 F.2d 1164 (Fed. Cir. 1993):

> [A] specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as in compliance with the enabling requirement of the first paragraph of § 112 *unless* there is reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support. (emphasis in original) 984 F.2d at 1171-1172.

In view of the foregoing, it is urged that the instant specification enables the full breadth of the claims and requests that the rejection under 35 U.S.C. 112, first paragraph, be withdrawn.

Rejection Under 35 U.S.C. § 102

The Examiner has maintained the rejection of Claims 1-6, 9-10 and 28 under 35 U.S.C. 102 (b) as being anticipated by Couraud et al.

It is well recognized in the art that antibodies react specifically with the antigen used as the immunogen. The remarkable specificity between a peptide and antibody depends on the three-dimensional structure of the peptidic backbone, the side chains, and their rotameric distributions, and the interaction of these epitopes with the hypervariable regions of the antibody. Harlow et al. (1999) notes that this specificity of interaction between antigen and antibody provides one of their most significant advantages in immunoprecipitation.

> Because monoclonal antibodies bind to only one epitope, they provide an excellent tool to identify a particular structure on an antigen. Given the right antibody, they can be used not only to detect an antigen, but also to distinguish among different forms of the antigen, including conformational changes or posttranslational modifications. (page 26)

Couraud et al., fragmented substance P and examined whether his anti-substance P antibody would react with the various fragments. Harlow et al. (1999) discusses fragmentation methods of epitope mapping at page 385.

> . . . these variants are effective, because only small amounts of protein need to be expressed to determine whether an antibody can bind to it. Thus, even if only a small fraction of the expressed protein fragment folds correctly, it may still be sufficient to give a strong antibody-binding signal, making this method useful for mapping the binding sites of antibodies to both conformation-sensitive and linear epitopes.

Applicant strongly disagrees with Examiner's statement (page 8 of Final Office Action) and urges that one skilled in the art would anticipate that the structure of antibody will vary

depending upon how the antibody is generated, specifically what immunogen is used to raise the antibody. Monoclonal antibodies recognize single epitopes on proteins. The epitope recognized by the monoclonal antibodies of Couraud et al., were characterized as conformationally based (page 1717, second column). Fragmentation of the intact substance P molecule by Couraud et al. and the absence of cross-reactivity of the fragment SP 8-11 (Table 3) suggests that the epitope recognized by the Couraud et al. monoclonal antibodies is likely formed by non-contiguous amino acids which were partially or wholly disrupted by the fragmentation process.

> Epitopes on an antigen can be formed either by a linear string of amino acid residues or by noncontinguous sequences that are folded into close proximity in the three-dimensional shape on the face of the antigen. (page 25)

As discussed more fully above, an epitope was recognized by one of skill in the art on the filing date of the instant application to be frequently composed of as few as 3 amino acids. Harlow 1999) The description of the monoclonal antibodies of Couraud, et al. viewed by a skilled artisan would not believe that antibodies raised to the peptides of SEQ ID NO:2 or SEQ ID NO:4 to be likely to have a similar structure. Furthermore, a skilled artisan would not expect am antibody raised to the peptides of the present invention (product-by-process) to be the same or obvious from the antibodies described in Couraud et al.

Use of the tetrapeptide and/or pentapeptide as the immunogen would be expected by one of skill in antibody art to generate antibodies having hypervariable regions distinct and apart from the antibodies described in Couraud et al.

According to section 2112 of the M.P.E.P.:

> The fact that a certain result or characteristic may occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic. *In re Rijckaert*, 9 F.3d 1531, 1534, 28 USPQ2d 1995, 1997 (Fed. Cir. 1993) (emphasis in original) (MPEP § 2112).

Further, the M.P.E.P. states that:

> To establish inherency, the extrinsic evidence 'must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. Inherency, however, may not be established by probabilities or possibilities. The mere fact that a certain thing may result from a given set of circumstances is not sufficient.' *In re Robertson*, 169 F.3d 743, 745, 49 USPQ2d 1949, 1950-51 (Fed. Cir. 1999)(MPEP § 2112)

Applicants respectfully submit that the Examiner has not met the burden of making it clear that the missing descriptive matter is necessarily present in Couraud et al. In fact, Couraud et al. is silent regarding the structure of the hypevariable regions of the listed antibodies. Applicants, therefore, submit that the Examiner has merely raised a possibility that the antibodies of Couraud et al. may be structurally similar to antibodies raised to the peptides of the present invention. As made clear by the M.P.E.P., such conjecture does not suffice as a finding that the prior art reference contains a disclosure that anticipates the presently claimed invention.

In addition, with regard to new Claims 32-34, Couraud et al., describes antibodies that do not cross-react with SP 8-11 and SP 7-11 (Table 3). Therefore, Couraud et al. cannot anticipate the antibodies of Claims 32-34.

In summary, the claims of the instant application are directed to isolated antibodies that are specific for and raised against the tetrapeptide and/or pentapeptides disclosed in the instant application. Couraud simply does not teach or suggest antibodies against these peptides. Thus, Couraud et al. does not anticipate or make obvious the claimed invention. Accordingly, withdrawal of the rejection under Section 102 (b) is respectfully requested.

Rejection Under 35 U.S.C. § 112

The Examiner has rejected Claim 31 under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The cancellation of Claim 31 obviates the rejection of this claim.

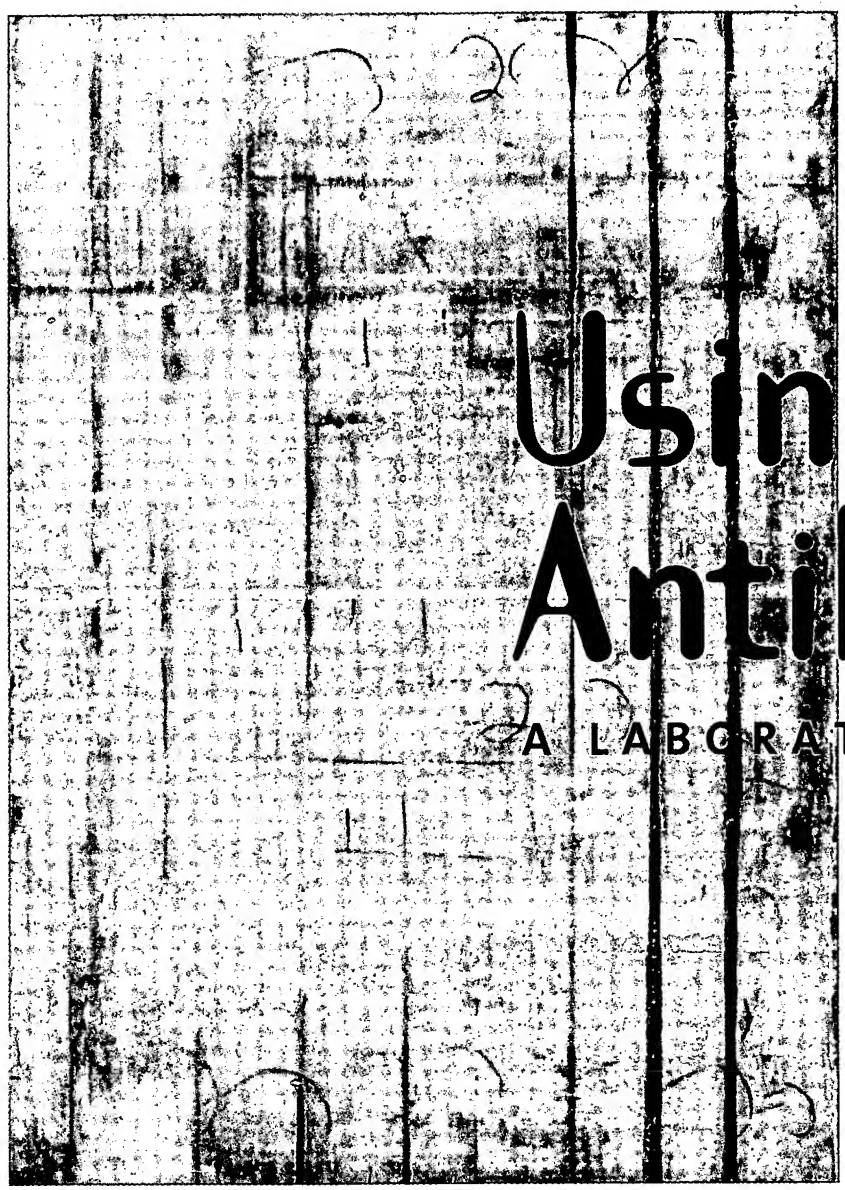
In view of the foregoing amendments and remarks, it submitted that the claims remaining for active consideration in this application are free of the cited art and in condition for allowance. Accordingly, favorable action at an early date will be appreciated. If the examiner is of the view that any issue remains unresolved, it is respectfully suggested that applicants= undersigned attorney may be contacted by telephone at the number set forth below.

Respectfully submitted,

By: _____

Nancy T. Morris, Reg. No. 42,017
STINSON MORRISON HECKER LLP
1201 Walnut Street, Suite 2800
Kansas City, MO 64106-2150
Direct Dial: (402) 930-1759
Facsimile: (816) 691-3495
Attorney for Applicant(s)

Enclosures



Using Antibodies

A LABORATORY MANUAL

Ed Harlow
MASSACHUSETTS GENERAL HOSPITAL CANCER CENTER
HARVARD MEDICAL SCHOOL

David Lane
DUNDEE UNIVERSITY

COLD SPRING HARBOR LABORATORY PRESS
Cold Spring Harbor, New York

Immunoprecipitations Using Polyclonal Antibodies

Polyclonal antibodies are the most commonly used reagents for immunoprecipitations. Normally they contain antibodies that bind to multiple sites on the antigen and therefore have a much higher avidity for the antigen (see Chapter 2). Having more than one antibody bound to an antigen also has other important advantages. When the immune complexes are collected on any of the solid-phase matrices, such as protein A beads, the availability of multiple binding sites for the protein A molecules provides a more stable antigen–antibody–protein A complex. Together, multiple antibody–antigen interactions and multiple antibody–protein A interactions provide a multivalent complex that is easy to prepare, stable, and can be treated relatively harshly during the washing procedure.

Although using polyclonal antibodies for immunoprecipitations often produces stable multivalent interactions, their use also yields higher nonspecific backgrounds than the use of other types of antibodies. Multiple interactions that lead to forming large complexes are more apt to trap or bind nonspecific proteins. Because polyclonal antibodies normally are used as whole sera, they contain the entire repertoire of circulating antibodies found in the immunized animal at the time the serum was collected. Therefore, serum may contain antibodies that specifically recognize spurious antigens. Because this type of contamination is specific, it cannot be removed by methods that are designed to lower nonspecific background (e.g., preclearing, adding BSA). In these cases, the easiest method to remove these activities is to switch antibody sources. Other antisera are unlikely to contain identical spurious reactions. In some cases, it may also be possible to block the specific antibodies by preincubating the serum with a solution that contains the contaminating proteins (e.g., an acetone powder from a source that does not express the antigen being studied, p. 437).

Because of contaminating activities and increased nonspecific interactions, immunoprecipitations using polyclonal antibodies normally have higher backgrounds than other antibody preparations. Many of these problems are inherent in this technique, but some of the background can be effectively removed by titrating the amount of antisera needed to immunoprecipitate the antigen. By providing the smallest amount of serum necessary for the quantitative recovery of the antigen, the background can be kept to a minimum. In addition, because of the stability of the complexes, nonspecific background problems may be lessened by more stringent washing.

Immunoprecipitations Using Monoclonal Antibodies

The biggest advantage of using monoclonal antibodies for immunoprecipitations is the specificity of their interactions. Because monoclonal antibodies bind to only one epitope, they provide an excellent tool to identify a particular structure on an antigen. Given the right antibody, they can be used not only to detect an antigen, but also to distinguish among different forms of the antigen, including conformational changes or posttranslational modifications. In addition, because the immune complexes formed using monoclonal antibodies are not usually multimeric and are smaller than

those formed when using polyclonal antibodies, there is less of a problem with nonspecific binding. Therefore, the backgrounds are normally cleaner.

Although using monoclonal antibodies for immunoprecipitations may solve or lessen some of the problems found when using polyclonal antibodies, their use also creates another set of difficulties. The most worrisome problem is affinity. Because the antigen is held only by one antibody–antigen interaction (except when the antigen is multimeric), the affinity of the antibody for the antigen is critically important (see discussions of affinity on p. 28). Monoclonal antibodies with affinities lower than about 10^8 liter mol^{-1} are difficult to use in immunoprecipitations. Because many screening techniques for hybridoma fusions detect antibodies with affinities as low as 10^6 liter mol^{-1}, not all monoclonal antibodies work well in immunoprecipitations.

A second problem with using monoclonal antibodies is the possibility of detecting spurious cross-reactions with other polypeptides. Because an epitope can be a relatively small protein structure, often composed of only 4 or 5 amino acids, there is a reasonable chance that a similar epitope can be found on another polypeptide. In some cases, the common epitopes form part of an important structural similarity between antigens, and monoclonal antibodies can be used to detect related antigens. Alternatively, the antibodies may detect small structural similarities confined only to the antibody combining site. This is particularly true for antibodies that recognize denaturation-resistant epitopes. Presumably this occurs because these antibodies recognize features found in the primary structure of the polypeptides. Depending on the set of hybridomas, as many as one in three monoclonal antibodies have been shown to display these types of cross-reactions. Because of the frequency of these cross-reactions, the precipitation of an unexpected polypeptide should be treated as a contaminant until proven otherwise.

Immunoprecipitations using pooled monoclonal antibodies

Using pools of monoclonal antibodies in immunoprecipitation takes advantage of the best properties of both polyclonal and individual monoclonal antibodies. The monoclonal antibodies provide specificity, and the use of multiple antibodies allows the formation of stable multivalent complexes. Consequently, pooled monoclonal antibodies are the best choice of reagents for most immunoprecipitations. Unfortunately, not all antigens have been studied in enough detail to have a set of monoclonal antibodies available for pooling. However, even the use of two antibodies specific for two separate epitopes will greatly increase the avidity for the antigen as well as for protein A or protein G. Therefore, whenever possible, pooled monoclonal antibodies should be used for immunoprecipitations.

Choosing an epitope-mapping method

There are many potential methods for mapping and characterizing the location of epitopes on proteins, ranging from solving the crystal structure of the antibody–antigen complex to analysis of vast libraries of random peptide sequences. These variants are discussed below. Three of the simplest, most widely applicable, and most robust assays are competition assays, gene expression assays, and synthetic peptide-based assays (Table 11.2).

Mapping by competition assay is a very widely used method that can rapidly determine whether two different monoclonal antibodies are able to bind independently to the same protein antigen or whether their binding sites on the protein overlap in such a way that both are not able to bind to the antigen at the same time. The assay can be configured in a large number of different formats using either labeled antigen or labeled antibody. Commonly, the antigen is immobilized on a 96-well plate. The ability of unlabeled antibodies to block the binding of labeled antibodies is measured using radioactive or enzyme labels.

Using this kind of assay with a panel of monoclonal antibodies allows the number of sterically discrete epitopes on the protein antigen to be determined. The method is very versatile and remarkably accurate. For example, we used this kind of assay to map 10 or more independent epitopes on SV40 large T antigen (Gannon and Lane 1990). It is particularly useful in determining if a new monoclonal antibody to a particular protein is distinct from other antibodies to the same protein (Wagener et al. 1983,

Table 11.2 *Application and requirements of recommended epitope-mapping methods*

Method	Conformational epitope	Linear epitope	Requirements	Precision
Competition assay	Yes	Yes	Labeled antibody; antigen	Determines steric competition only
Gene fragment expression	Often but not always	Yes	cDNA must be cloned (gene sequence known)	Conformational 50–200 amino acid domains. Linear 10–20 amino acids
Synthetic peptide library	Never	Yes	Peptide library must be made but cDNA clone not required	Complete description of epitope 3–15 amino acids

1984; Kuroki et al. 1990, 1992a,b). The assay can be used with antibodies that bind both conformational and linear epitopes.

The second approach to mapping an epitope is based on the concept of cutting the protein into smaller fragments and then examining whether the antibody will react with any of these pieces. Historically, these fragments were produced by chemical or proteolytic cleavage of the protein antigen. These remain powerful methods, but the advent of systems for the expression of recombinant proteins has allowed an alternative genetic approach to protein fragmentation. In these procedures, the open reading frame encoding the protein is fragmented either randomly or by specific genetic construction, and the reactivity of the expressed fragments of the protein with the test antibody is determined.

The versatility of DNA fragmentation protocols combined with the vast range of available systems for the expression of recombinant protein has created an almost infinite number of variants on this theme. These range from the entire synthesis of gene segments (Alexander et al. 1992) to the cloning of random fragments of the open reading frame generated by digestion with DNase for expression on the surface of bacteriophage particles (Petersen et al. 1995; Fack et al. 1997).

All of these variants are effective, because only small amounts of protein need to be expressed to determine whether an antibody can bind to it. Thus, even if only a small fraction of the expressed protein fragment folds correctly, it may still be sufficient to give a strong antibody-binding signal, making this method useful for mapping the binding sites of antibodies to both conformation-sensitive and linear epitopes.

A useful example protocol where defined gene fragments are produced by PCR and then transcribed and translated into protein in vitro in the presence of radioactive amino acids is outlined below (p. 391). Binding of the antibody to the labeled protein fragments is then determined by immunoprecipitation and gel electrophoresis.

The third approach to epitope mapping is only applicable to antibodies that work in immunoblotting and react with short linear peptide epitopes. The identification of the epitopes with which these antibodies react has been done using large libraries of random peptide sequence displayed on the surface of phage particles. Alternatively, vast libraries of random synthetic peptides have been analyzed. A much simpler approach in cases where the amino acid sequence of the protein or gene fragment contains the epitope is to synthesize (or order) a defined library of overlapping peptide segments of the protein. These peptide set libraries can then be easily tested for binding to the test antibody in simple binding assays and will define the linear epitope to a stretch of 5–15 amino acids.

The region of an antigen that binds to an antibody is called an epitope

The region of an antigen that interacts with an antibody is defined as an epitope. An epitope is not an intrinsic property of any particular structure, as it is defined only by reference to the binding site of an antibody. The size of an epitope is governed by the size of the combining site. From X-ray studies of the structures of cocrystals between small antigens bound to antibodies, the size of the combining site was thought to be relatively small. The site was visualized as a cleft or pocket into which the epitope docked. Relatively few of the amino acid side chains of the CDR were in close contact with the antigen. Later work using larger antigens showed that the area of these antigens in close apposition to the antibody can be quite large, occupying as much as 500–750 $Å^2$ and often involving contacts with multiple CDRs, and many times establishing contact with all six. Although these studies have shown that epitopes can be much larger than originally thought, it is still clear that high-affinity antibodies can be raised to small epitopes.

Because antibodies recognize relatively small regions of an entire antigen, occasionally they can find related structures on other molecules. This forms the molecular basis for cross-reaction. Cross-reactions can be helpful in finding related protein family members or distracting when they recognize unrelated proteins with a shared structural feature. For example, cross-reactions can detect highly related structures in common structural regions of protein family members. In this way, an antibody can be a useful tool to identify and study related proteins. However, cross-reactions may also detect similar spatial features in other antigens that do not represent shared structural domains. In these cases, the interactions may still be quite strong but the resulting interactions distracting rather than helpful. Therefore, it is always important to interpret cross-reactions in a conservative manner. Keep in mind that the presence of similar epitopes does not necessarily imply a functional relationship.

Epitopes on protein antigens are local surface structures that can be formed by contiguous or noncontiguous amino acid sequences

Epitopes on an antigen can be formed either by a linear string of amino acid residues or by noncontinguous sequences that are folded into close proximity in the three-dimensional shape on the face of the antigen (Fig. 2.2). A good example of this is seen with one of the lysozyme–antibody cocrystals. Here, the amino acids of lysozyme that form the epitope come from two distant stretches of the primary sequence (residues 18–27 and residues 116–129). Although separated from each other in the primary sequence, these stretches of amino acids are adjacent on the protein surface. At the interface between the antigen and the antibody, a total of 16 amino acids of the antigen make close contacts with 17 amino acids of the antibody, the latter involving all six CDRs. The whole interface is tightly packed and excludes solvent. Strikingly, 748 $Å^2$ or 11% of the surface of lysozyme is covered by the antibody. Similar conclusions come from the study of the second lysozyme–antibody cocrystals and the neuraminidase–antibody cocrystals. Here, either three (lysozyme) or four (neuraminidase) stretches of distant primary sequence form portions of the epitope structure.

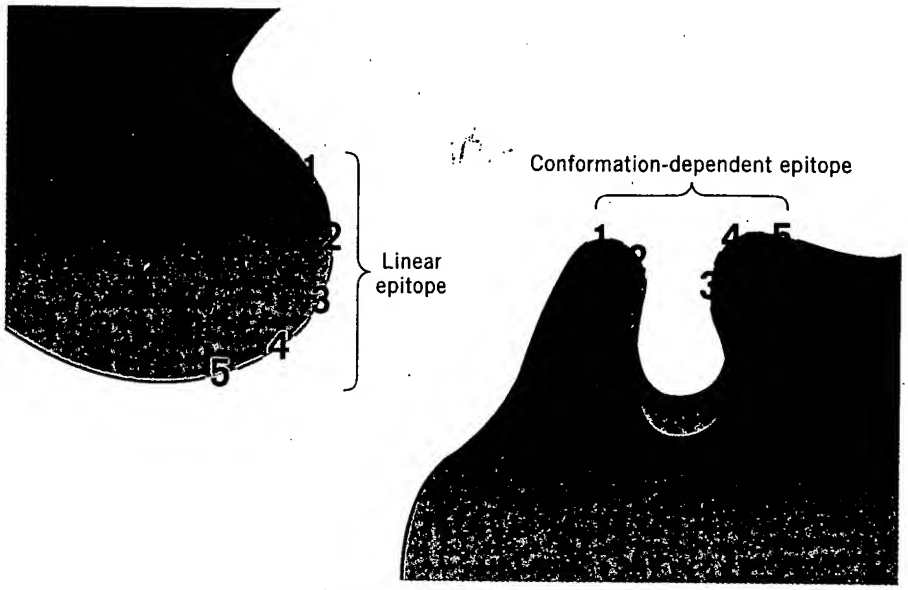


Figure 2.2
Epitopes on an antigen can be formed either by a linear string of amino acid residues or by non-contiguous sequences that are folded into close proximity in the three-dimensional shape on the face of the antigen.

Work with antibodies raised against synthetic peptides or other small antigens provides a set of excellent examples for interactions between antibodies and small, well-defined epitopes. One set of commonly used antibodies that display this property are the anti-phosphotyrosine antibodies, which specifically recognize the phosphorylated side chain of this amino acid in different local regions of many proteins. The ability of antibodies to recognize small epitopes in various structural environments shows the versatility of antibodies to recognize small discrete regions.

Some immune complexes show no alterations in the structure of the antibody or antigen, whereas others show large conformational changes

Antibody–antigen interactions can occur either with large structural changes in the antibody or the antigen or with no detectable changes. From the structures of the first antibody–protein antigen cocrystals, it was clear that both flexible and rigid structures can form good epitopes. In the crystal structure of one of the lysozyme–antibody complexes, no distortion of either the antigen or antibody could be detected, even at high resolution. In sharp contrast, the crystal structure of a neuraminidase–antibody complex revealed substantial structural alterations of both the antigen and antibody. Because the crystallization process itself can induce structural alterations, it is difficult to

prove that these changes are due to antibody binding. However, many other studies have shown that antibodies can induce structural changes in antigens. Good examples of this are the removal of heme from myoglobin and the activation of enzymes by antibody binding.

The antibody–antigen complex is held together by multiple noncovalent bonds

The binding of the antibody to the antigen is entirely dependent on noncovalent interactions, and the antibody–antigen complex is in equilibrium with the free components. The immune complex is stabilized by the combination of weak interactions that depend on the precise alignment of the antigen and antibody. These noncovalent interactions include hydrogen bonds, van der Waals forces, coulombic interactions, and hydrophobic interactions. These interactions can occur between side chains or the polypeptide backbones.

Small changes in antigen structure can affect profoundly the strength of the antibody–antigen interaction. The loss of a single hydrogen bond at the interface can reduce the strength of interaction 1000-fold. The overall interaction is a balance of many attractive and repulsive interactions at the interface. This can be demonstrated in vitro by site-directed mutagenesis. Changing the amino acid residues that form the binding site can alter the strength of an antibody–antigen interaction. This is performed elegantly in vivo by the selection of cells secreting higher-affinity antibodies. By a still poorly understood process, the CDR residues from differentiating clones of B cells undergo extensive mutation, yielding antibodies that differ widely in the microstructure of their antigen-binding sites. Cells that express antibodies with higher affinity are stimulated preferentially to divide. This process continues during the exposure and reexposure to antigen and results in a stronger and more specific antibody response.

Antibodies can bind to a wide range of chemical structures and can discriminate among related compounds

The microenvironment of the combining site can accommodate highly charged as well as hydrophobic molecules. Epitopes composed of carbohydrates, lipids, nucleic acids, amino acids, and a wide range of synthetic organic chemicals have all been identified. The repertoire of possible binding sites is enormous, and antibodies that are specific to novel compounds can be derived readily.

The specificity of antibodies has been demonstrated by a large number of experiments showing that small changes in the epitope structure can prevent antigen recognition. For example, antibodies have been isolated that will differentiate between conformations of protein antigens, detect single amino acid substitutions, or act as weak enzymes by stabilizing transition forms.